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Comparing the secretome response of *Aspergillus* and *Fusarium* species on chemically treated plastics

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INTRODUCTION & AIM

Plastic pollution is one of the most pressing environmental issues of our time, with millions of tons of plastic waste accumulating in landfills and natural ecosystems every year. Among these, polyethylene terephthalate (PET) and polylactic acid (PLA) are widely used polymers that persist in the environment due to their slow degradation rates [1], [2]. PET is extensively used in packaging, textiles, and plastic bottles, while PLA is a biodegradable polymer commonly found in compostable plastics. However, the natural degradation of these materials is inefficient and requires innovative biotechnological solutions. In recent years, microbial degradation of plastics has gained attention as a sustainable alternative for managing plastic waste [3]. Fungi have shown significant potential due to their ability to produce extracellular enzymes capable of breaking down complex polymers [4]. This study aims to screen fungal strains for their ability to degrade PET and PLA and to evaluate the enzymatic activity involved in this process.

METHODS

Materials & Polymer Preparation

BHET and casein were purchased from Sigma-Aldrich. PLA (Ingeo™ 3001D) was hydrolyzed at 60 °C for 360 h and and PET (PoliPET™ 76W) was glycolyzed with ethylene glycol at 180 °C for 6 h, to obtain oligomers used in degradation assays (Table 1).

Fungal Cultivation

Fungal strains were grown on YPD agar (30 °C, 3 days). Plugs were transferred to mineral medium (MM) for liquid culture screening, with either 1% casein, 1% olive oil or 1% BHET as inducers.

Screening for Plastic Degradation

Initial screening was performed on MM-agar plates containing 1% PLA or PET oligomers. Strains with clearance zones and growth were selected for further testing in liquid cultures with plastic oligomers as the sole carbon source.

Enzyme Activity Assays

Protein concentration was measured by Bradford assay [5]. Esterase activity was tested with pNPA, and protease activity was measured using casein as substrate.

Proteomic Sample Preparation

Supernatants from high-activity cultures (PLA + casein-induced) were filtered, concentrated, dialyzed, and freeze-dried before mass spectrometry analysis.

RESULTS & DISCUSSION

Plate screening for PET/PLA degraders

- Among the strains tested on PET dispersion plates, *Fusarium oxysporum* BPOP18 and *Aspergillus parasiticus* MM36 showed visible growth. This was observed as dense mycelial biomass and distinct clearance halos around the colonies (Fig. 1A, B)
- The same two previous ones exhibited visible growth on PLA dispersion. This growth was also observed as the formation of mycelial biomass on the agar plates (Fig. 2A, B).

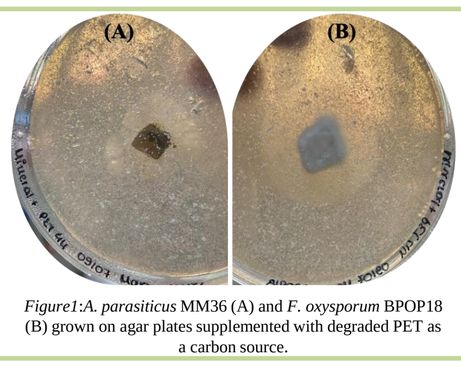

Figure1:*A. parasiticus* MM36 (A) and *F. oxysporum* BPOP18 (B) grown on agar plates supplemented with degraded PET as a carbon source.

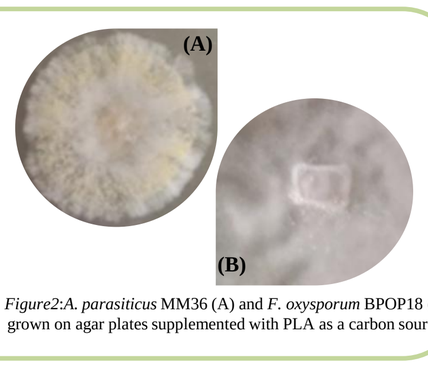

Figure2:*A. parasiticus* MM36 (A) and *F. oxysporum* BPOP18 (B) grown on agar plates supplemented with PLA as a carbon source.

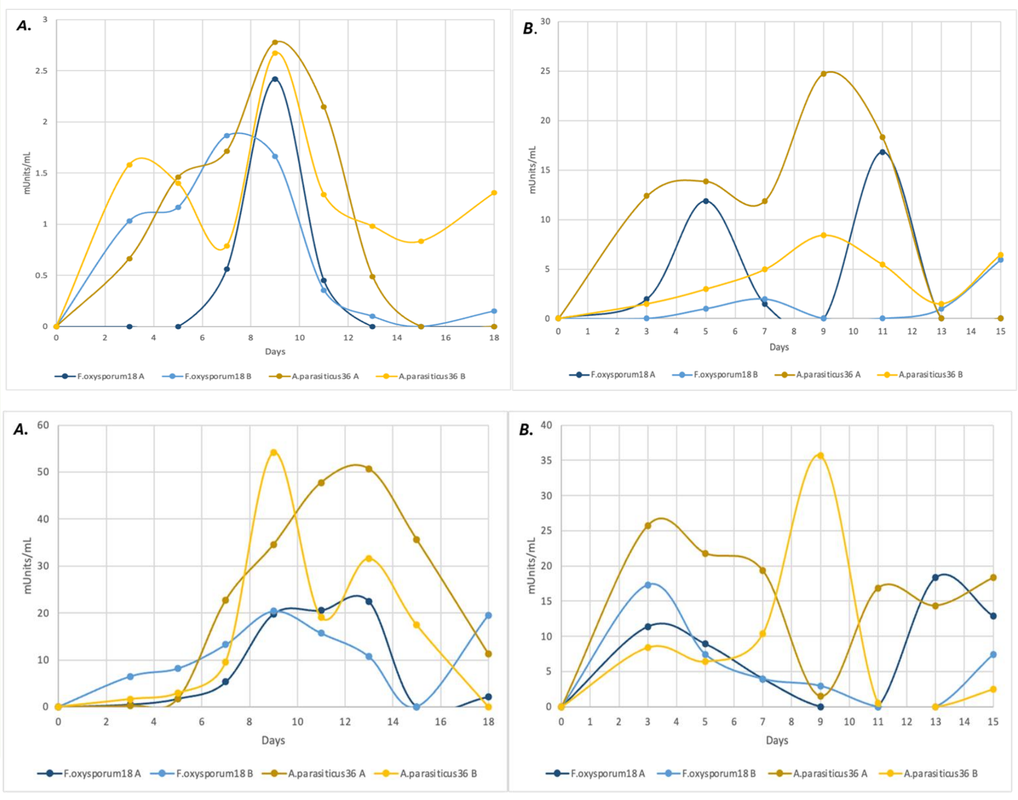

Figure 3: Time course of esterase (A1, A2) and protease (B1, B2) activity of in fungal cultures grown with degraded PLA, with biomass pre-grown on olive oil (A1, B1) and casein (A2, B2)

Time-course of enzyme expression in fungal cultures

Cultures with degraded PET:

- No activity was detected in cultures pre-grown with BHET,
- Olive oil-based precultures induced only slight esterase activity.

Cultures with degraded PLA

Figure 3 presents the time course of esterase (left) and protease (right) activity in fungal cultures grown with degraded PLA, with biomass pre-grown on olive oil and casein, respectively.

- Low enzymatic activity suggests that olive oil does not efficiently induce the expression of enzymes involved in PLA degradation.
- When grown in casein, both enzymatic activities were significantly higher compared to cultures pre-grown with olive oil.

Material	$\overline{M_n}$ (g/mol)	$\overline{M_w}$ (g/mol)	x_c (%)
Virgin PET	35,500	-	36
Degraded PET	10,700	-	42
Virgin PLA (3001D)	61,000	94,000	46
Degraded PLA (3001D)	3,200	7,800	76

Table 1. *Molecular weights and crystallinity degree of PET and PLA before and after solvolysis.*

Proteomic Profiling Reveals Functional Enzyme Shifts in PLA-Grown Cultures of F. oxysporum BPOP18

Only cultures grown on degraded PLA from casein precultures were selected for proteomic analysis due to higher enzyme activity and protein concentration. A comparative proteomic analysis was performed against **lactic acid (LA)** as control (Fig. 5). The scatter plot (Fig. 4) shows protein abundance of *F. oxysporum* BPOP18 cultures in PLA (Y-axis) versus log-fold change (logFC) relative to LA (X-axis), with proteins coloured by functional category.

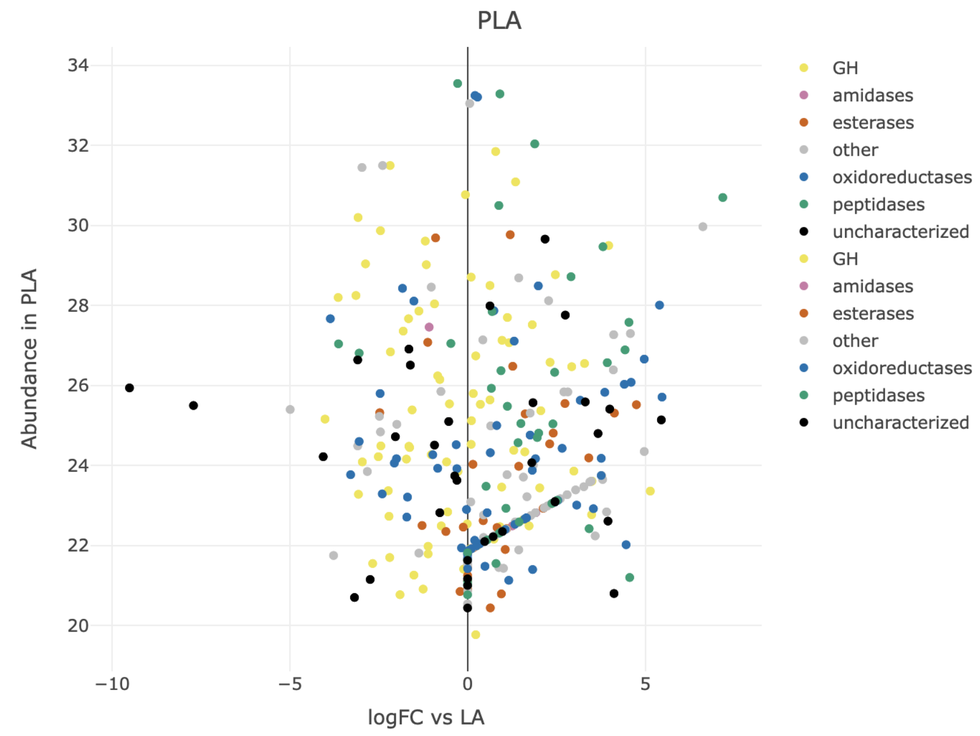

Figure 4: Protein abundance in PLA (Y-axis) versus log-fold change (logFC) relative to LA (X-axis), with proteins coloured by functional category

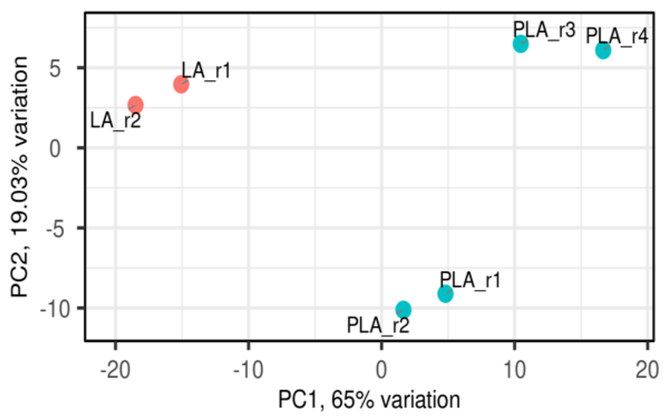

Figure 5: Biplot of the first two principal components (PCs) of protein intensities

CONCLUSIONS

This study highlights the potential of fungi species in plastic biodegradation, with a focus on PLA degradation.

- Plate screening identified strains capable of growing on both PET and PLA oligomers.
- Enzyme assays confirmed extracellular activity linked to polymer breakdown.
- Focusing on *F. oxysporum* BPOP18, proteomic analysis of PLA-grown cultures revealed a selective upregulation of a plethora of proteins.
- Further analysis of these proteomic datasets will support the **selection and functional validation of candidate enzymes** involved in PLA degradation, paving the way for targeted **enzymatic depolymerization strategies**.

- **Upregulation of some glycoside hydrolases (GHs)**, **oxidoreductases**, as well as **esterases.**
- A subset of **peptidases** are more abundant in PLA.
- Several **uncharacterized proteins** are upregulated.

The distribution indicates a **broad but selective proteomic response**, with a shift toward enzymes possibly involved in **PLA degradation or stress adaptation**.

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